Radiations and biodegradation techniques for detoxifying *Carica papaya* seed oil for effective dietary and industrial use

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Abstract Benzyl isothiocyanate (BITC) is toxic in high concentration. The capacity of *Aspergillus niger*, microwave and ultraviolet radiations to reduce the BITC levels in *Carica papaya* Linn seed oil were assessed *in vitro*. BITC at different concentrations were periodically exposed to microwave and ultraviolet radiations for 30 min and 10 h, respectively; and to identify *Aspergillus niger* for 4 days. Microwave radiation significantly reduced ($p < 0.05$) BITC levels (0.0272, 0.0544, and 0.0816 μmol) to 12.19, 8.99 and 27.5 % respectively within 15 min. Ultraviolet radiation significantly reduced ($p < 0.05$) BITC levels at all the concentrations. *A. niger* significantly increased ($p < 0.05$) BITC degradation on days 2 and 4 at 0.816, 1.36 and 2.72 nmol. Glutathione activity was significantly increased ($p < 0.05$) while glutathione S-transferase activity significantly reduced ($p < 0.05$) at all concentrations on days 3 and 4 respectively. The three techniques are possible models for improving the dietary consumption of the oil.

Keywords *Carica papaya* seed · Benzyl isothiocyanate · *Aspergillus niger* · Microwave · Ultraviolet light

Research highlights

- Three techniques were developed for detoxification of *Carica papaya* seed oil.
- *Aspergillus niger* was first detected to degrade benzene isothiocyanate.
- Microwave and ultraviolet radiations were first detected to degrade the compound.
- The culinary use and commercial value of the health beneficial oil may be enhanced.

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Introduction

Carica papaya Linn that belongs to a small family Caricaceae, is generally recognized for its food and nutritional values all over the world (Krishna et al. 2008). The quantity of papaya produced in Nigeria is projected to be 765,000 metric tonnes yearly and this makes Nigeria to be one of its largest producers (FAO 2007). Aqueous and organic extracts of the seeds have anti-helminthic activity against *Caenorhabditis elegans* (Kermanshai et al. 2001), prevent the growth of trichomoniasis (Calzada et al. 2007), and *Escherichia coli* (Krishna et al. 2008). Nigeria is a unique country with a diversity of oil seeds (Afolabi 2008). Exploitation of new avenues of oil in order to complement the existing ones is a growing concern (Ikhuoria et al. 2008). The major challenge with the usage and benefits of these oils in Nigeria lies in the area of absence or inadequate information on the exploitation and characteristics of the diverse oil seed plants (Ikhuoria et al. 2008).

The papaya seeds largely been thrown away as garbage after the fruit is consumed despite its health benefits. The detail nutritional and fatty acids composition were earlier accounted for (Puangsri et al. 2005). Research efforts are on the increase to improve the efficient usage of papaya, and the utilization of its seeds (Afolabi et al. 2011b). Research has reported the seed to be an extremely good source of oil (25.6 %), which may be beneficial for medicinal, biofuel, and industrial purposes (Afolabi et al. 2011b). It has been confirmed that oil extracted from the seeds is rich in antioxidants and has a high oxidative stability, which is a good indicator of resistance to lipid peroxidation during storage (Afolabi and Ofobrukeweta 2011). The oil is high in oleic fatty acids, low in poly unsaturated fatty acids and contains phenolic compounds that have antioxidant activity, which are beneficial to health (Afolabi and Ofobrukeweta 2011). These properties would make the oil an acceptable alternative for other highly unsaturated oils. Papaya seed oil may be considered as

a healthy substitute in the food industry because of its richness in oleic oil, which has chemo-protective properties. Papaya seed oil was also reported to be of better health benefits than the commonly consumed groundnut oil (Afolabi et al. 2011a).

There is no part of the papaya plant that may be considered unimportant due to their nutritional, therapeutic and medicinal benefits (Sofrata et al. 2011). The latter two benefits had been attributed to the presence of sulfurous compounds with chemopreventive properties known as benzyl isothiocyanate (Cowan 1999; Kim et al. 2013; Lohiya et al. 2008; Pedro et al. 2011), The level ranging from 4.0 to 23.3 g/kg depending on the various processing methods for the pre-treatment of papaya seeds (Kermanshai et al. 2001; Lee et al. 2011). BITC is also found to have cancer preventive properties by controlling phase I drug-metabolizing enzymes and phase II detoxification enzymes (Kim et al. 2013; Zhang et al. 1992). However, its presence in extremely large quantities contributes to the limited culinary uses of the seed oil. BITC has been found to be toxic to cells at large concentrations, causing cell necrosis (Temminck et al. 1986), irreversible degeneration of smooth muscle, temporary sterility (Lohiya et al. 2008) and renal dysfunction. Puangsri et al. (2005) reported the likelihood of improving the variability of the oil through enzymatic modification with the aim of making it greatly desirable to the food and culinary industries. The benzyl isothiocyanate content present therein must be reduced for papaya seed oil to be developed into edible oil.

Irradiation has been recognized as a safe and effective method for food preservation among all existing technologies (Bangash et al. 2007). The stability of canola, sunflower, neem, castor and chicken oils have been improved at a varying degree by irradiation treatment (Bangash et al. 2007; Kumar and Viswanathan 2013). The use of *Aspergillus niger* and the components of electromagnetic radiation for BITC degradation have never been reported before. This work was therefore designed to establish the effectiveness of biodegradation, microwave and ultraviolet radiation methods for reducing BITC level in the oil so as to increase the culinary uses of the oil.

Materials and methods

Chemicals

Benzyl isothiocyanate (BITC) and 1,2-benzenedithiol used for this study were products of Sigma Aldrich Incorporation (USA) and Georganics Ltd (Slovakia) respectively. All chemicals used were of analytical grade.

Collection and processing of seeds

The seeds of mature ripe fruits of *C. papaya* Linn (FHI108906) were collected from the fruit sellers within

Covenant University environ in Ota, Ogun State of Nigeria. The fruit was identified in the Applied Biology unit of the Biological Sciences Department at Covenant University. It was later deposited in the Hebarium unit of the Forest Research Institute of Nigeria (FRIN), Ibadan, Nigeria, for reconfirmation. The seeds harvested from papaya fruits were oven dried at a temperature of 45–50 °C for 48 h. The dried seeds were blended into powdered form using the dry program of a blender (Emel EM-242) and stored in clean air tight container prior to use. Oil was extracted from the seeds using a mixture of methanol and diethyl ether (70:30) as solvent in a Soxhlet apparatus. Higher proportion (70 %) of polar solvent (methanol), which is one of the recommended solvent for extraction of vegetable oil, was used to improve oil yield and physical quality of the recovered oil (Brito Mariano et al. 2011; Nwabueze and Okocha 2008). Also, 30 % of diethyl ether was incorporated as co-solvent to increase the solubility of BITC, which is a non-polar compound. The oil extract was then subjected to pure recovery using a Stuart rotary vacuum evaporator (RE 300) at 50 °C yielding fine, clear golden yellow coloured oil with moisture content of 0.223 %.

Exposure of samples to microwave and ultraviolet radiations

BITC sample solutions of 0.0272, 0.0544, 0.0816, 0.1088, and 0.136 µmol concentrations from earlier prepared BITC stock solution (1.36 µl of BITC in 10 ml dimethyl sulfoxide (DMSO)) were dispensed into glass screw top vials. The samples were exposed to the microwave radiations for duration of 2, 5, 15 and 30 min, and ultraviolet radiations for duration of 2, 4, 6, 8 and 10 h. The 30 min exposure period of the oil to microwave and 10 h used for the ultraviolet light were informed by the time BITC level could not be detected by our analytical method in our preliminary work. The microwave was generated with 800 W compact Sanyo microwave oven (EM-51055 UK2, China) having pre-set the regulator to medium-low. The ultraviolet radiation was generated with a Lab caire laminar flow (PCR8-075, England). Samples that were not exposed to microwave radiations and ultraviolet radiations served as control for their appropriate experiment. All analysis was carried out in duplicates.

Screening and identification of microorganism survival

The modified method of Khan and Priya (2011) was used. Oil extracted from the seeds was used to enrich soil. Some of the soil sample was collected 4 days later with a sterile spatula. Serial dilution was performed by adding 1.0 g of the soil to 10 ml of distilled water. A series of dilution of the suspension from 10^0 and 10^{-5} was carried out. An aliquot of 1.0 ml of the soil suspension was layered on petri dishes containing sterile potato dextrose agar (PDA) and the oil sample. All the plates were incubated at 25–27 °C for 5 days. Isolates were identified

and pure culture was maintained on PDA slants. The organisms were identified with the aid of an identification manual by Barnett and Hunter (1972). Fungal spore count was also carried out.

Cultivation medium

Yeast extract powder supplemented with sucrose was used as the broth medium. Aliquots of the broth medium (25 ml) were distributed into six sets of Erlenmeyer flasks. BITC sample solutions of 0.136, 0.408, 0.816, 1.36 and 2.72 nmol concentration from earlier prepared stock solution (1.36 μl of BITC in 10 ml dimethyl sulfoxide (DMSO)) were dispensed into the Erlenmeyer flasks containing the broth medium. The concentration of BITC sample solutions used for the cultivation medium was in nmol. This is because earlier experiments carried out using μmol did not allow the growth of *Aspergillus niger*, due to the purity of the BITC standard. BITC was not added to control. *A. niger* culture that was maintained on PDA slants at 25–27 °C for 5 days was used in this experiment. Cell suspension of *A. niger* was transferred to the flasks containing the broth medium and varying concentration of BITC. The flasks were monitored daily and observations were recorded.

Analysis of fungal mycelia

A flask was removed from each set at 24 h interval and the mycelia immediately separated. Mycelia (1 g) was collected by filtration with distilled water and then homogenized with 2 ml of 0.02 M Tris-HCL buffer, pH 7.0 containing 2 mM mercaptoethanol. The homogenate was centrifuged at 12,000 rpm for 15 min and the supernatant was used for other assays. This procedure was repeated daily.

Determination of BITC concentration in cultivation media

The modified method of Zhang et al. (1992) was used. The cultivation media was separated and 50 μl of it was added to screw top glass vials containing 450 μl of 100 mM potassium phosphate buffer, pH 8.5 and 450 μl of DMSO. An aliquot of 50 μl of 13.3 mM 1, 2-benzenedithiol was added last to initiate the conversion of isothiocyanate into 1, 3-benzenedithiole-2-thione. The mixture was heated for 1 h at 65 °C in a water bath and allowed to cool at room temperature for 5 min before the absorbance was read at 365 nm. All the reactions were carried out in duplicates.

Procedure for assay of glutathione s-transferase (GST) activity

GST catalytic activity was determined spectrophotometrically with the aromatic substrate 1-Chloro-2,4-dinitrobenzene (CDNB) by monitoring the changes in absorbance at 340 nm as described by Habig et al. (1974). An aliquot of

50 μl of 1.0 mM GSH, 50 μl of 1.0 mM CDNB and 1000 μl of 0.1 M Phosphate buffer, pH 6.5 were added to make up to a volume of 1.10 ml. The reaction was started by the addition of 50 μl of the supernatant obtained from the mycelia to this mixture. The increase in absorbance at 340 nm was monitored against a control blank containing 0.1 M Phosphate buffer, pH 6.5 instead of the enzyme, which was treated similarly as experimental samples. Readings were recorded every 60 s for a period of five minutes. The product extinction coefficient was taken to be 9.6 $\text{mM}^{-1} \text{cm}^{-1}$ (Habig et al. 1974). One unit of GST activity was defined as the nmoles of CDNB conjugated per minute. Protein concentration was determined by the method of Bradford (1976) using bovine serum albumin (BSA) as a standard. All analysis was carried out in duplicates.

Determination of cellular glutathione

Reduced glutathione (GSH) levels in control and BITC treated samples were measured by the method of Ellman (1959). Cell lysates were treated with 10 % trichloroacetic acid (150 μl) to precipitate proteins. The treated sample was centrifuged for 15 min at 30,000 rpm. An aliquot of 200 μl of the supernatant was diluted with 400 μl of 0.4 M Tris buffer, pH 8.9. Freshly prepared 5, 5'-dithiobis (2-nitrobenzoic acid) reagent in methanol (10 μl) was added to start the reaction and the absorbance was read at 412 nm within 5 min. The extinction coefficient was taken to be 14.15 $\text{mM}^{-1} \text{cm}^{-1}$ (20). The concentration was calculated using the formula:

$$\text{GSH (mM)} = \text{Absorbance} / (14.15 \times 2.05 \times \text{dilution factor})$$

Statistical analysis

One factor randomized complete block design was used for this experiment. Data were analyzed using analysis of variance (ANOVA) MegaStat statistical software package supplied by Dataville Solutions Ltd. (Lagos, Nigeria). Treatments with a *P* value of <0.05 were considered statistically significant and were subjected to a Student *t*-test using the same statistical software. The results were expressed as mean $\pm$ standard deviation.

Results

Use of microwave and ultraviolet radiations for BITC degradation

Effect of microwave radiations on BITC

Microwave treatment significantly reduced ($p < 0.05$) the transformation of 0.0272 μmol BITC to 1,3-benzodithiole-2-

thione throughout the 30 min exposure period (Fig. 1). Similar significant reduction ($p<0.05$) in the transformation of 0.0544 and 0.0816 μmol of BITC to 1, 3-benzodithiole-2-thione occurred within 5–30 min exposure period (Fig. 1). Microwave treatment had no significant effect ($p<0.05$) on 0.1088 and 0.136 μmol of BITC throughout our study duration.

Effect of ultraviolet radiations on BITC

Ultraviolet radiation significantly reduced ($p<0.05$) the transformation of 0.0272 and 0.0816 μmol BITC to 1, 3-benzodithiole-2-thione during exposure period of 6–10 h (Fig. 2). Exposure of 0.0544 and 0.1088 μmol BITC to ultraviolet radiations significantly reduced ($p<0.05$) the transformation of BITC to 1, 3-benzodithiole-2-thione within 2–10 h (Fig. 2). The ultraviolet radiation significantly reduced ($p<0.05$) the 0.136 μmol of BITC only at 10 h exposure period.

Use of biodegradation techniques for BITC degradation

A mixed culture of microbes was detected from the serial dilution carried out on the soil sample enriched with papaya seed oil; however, *Aspergillus niger* was the most predominant survival organism on the oil. No growth of *A. niger* was seen in all the samples including the control on day 0. The same trend continued on day 1 except that the control appeared to be cloudy. *A. niger* growth increased in the control and sample treated with 0.136 nmol of BITC on day 2. *A. niger* growth also increased in the control and samples treated with 0.136, 0.408 and 0.816 nmol of BITC on 3. *A. niger* growth increased in the control and in all the BITC treated samples on day 4.

There was a significant increase ($p<0.05$) in the conversion of BITC to 1,3-benzodithiole-2-thione at the concentration of 0.816, 1.36 and 2.72 nmol at days 2 and 4 (Fig. 3). The BITC compositions at 0.408, 0.816, 1.36 and 2.72 nmol significantly reduced ($p<0.05$) GST activity of *A. niger* on day 3. All the BITC compositions in general, significantly reduced ($p<0.05$) GST activity of *A. niger* on day 4 (Fig. 4). All the BITC compositions significantly increased ($p<0.05$) cellular GSH level of *A. niger* on day 3 (Fig. 5).

Discussions

Benzyl isothiocyanate (BITC) is a major bioactive compound that contributes to the medicinal values of the seed oil. The culinary uses of the seed oil are limited by the presence of the compound in extremely large quantities. The levels of BITC transformed to 1, 3-benzodithiole-2-thione was used to monitor the amount of BITC available in the samples after exposure to the three techniques (microwave radiation, ultraviolet radiation and *A. niger*) used in this study.

Use of microwave and ultraviolet radiations for BITC degradation

BITC was exposed to two different types of radiation. Microwave-ultraviolet radiation combination had earlier been used to degrade a similar organic compound (humic acid) through a thermal rearrangement of 2-benzyloxyacetophenone in the presence of aluminum pillared bentonite, which gives rise to 1-(o-hydroxyphenyl)-3-phenyl propane-1,3-diones (Zhang et al. 1992). Exposure of the varying concentration of BITC (0.0272, 0.0544, 0.0816, 0.1088 and 0.136 μmol) to

Fig. 1 Effect of microwave radiations on benzyl isothiocyanate

Fig. 2 Effect of ultraviolet radiations on benzyl isothiocyanate

microwave radiations led to their degradation within a period of 2–30 min. The more the time of exposure to microwave radiation, the more the quantity of BITC degraded (Fig. 1). The microwave radiation completely eliminated BITC at all concentration used in this study at 30 mins treatment (Fig. 1). It succeeded in eliminating BITC at 0.0272 μmol concentration by 69.9, 86.5 and 87.8 % within 2, 5, 15 and 30 mins treatment. Similar trend was noticed at other concentration. 38.3, 55.0 and 91.0 % of the BITC was effectively eliminated at 0.0544 μmol concentration; while 33.3, 73.04 and 72.47 % was eliminated at 0.0816 μmol concentration. This rapid action of microwave radiation during food processing with the advantages of uniform and mild heating that leads to minimal nutrient loss and improved quality was earlier reported (Afolabi 2014;

Azadmard-Damirchi et al. 2010; Clary et al. 2007; Garcia-Salas et al. 2010; Schulze et al. 2014).

BITC degradation followed this similar trend when exposed to ultraviolet radiations (Fig. 2). The BITC degradation rate observed from Fig. 2 under the ultraviolet radiation occurred at 18.86, 19.11, 25.31, 18.91 and 22.355 nmol/h for 0.0272, 0.0544, 0.0816, 0.1088 and 0.136 μmol respectively. Ten hours seem to be sufficient for the ultraviolet radiation to remarkably remove BITC at all the concentrations used in this study (Fig. 2). The levels of BITC transformation to 1, 3-benzodithiole-2-thione occurred at a slower rate compared to that of the microwave radiation. Ultraviolet radiation was most effective at 10 h treatment in eliminating between 68.7 and 97.5 % of BITC at all concentration used in this study

Fig. 3 Levels of benzyl isothiocyanate biotransformation to 1,3-benzodithiole-2-thione by *A. niger*

Fig. 4 The effect of benzyl isothiocyanate on Glutathione S-Transferase activity in *A. niger*

(Fig. 1). Generally, varying degradation levels (2.5–72.3 %) were attained prior to the 10 h of treatments, with obvious peak degradation levels of 72.4 % at 2 h for 0.1088 μmol concentration, and 59.9, 72.4, 67.5 and 50 % at 8 h for 0.0272, 0.0544, 0.0816 and 0.136 μmol concentrations. Thus, eliminating BITC content with treatment with ultraviolet radiations generally becomes effective with increasing length of exposure period. Similar elimination of harmful perchlorate compound during water treatment by ultraviolet radiation was earlier reported (Vellanki and Batchelor 2013). Ultraviolet radiation was also reported to preserve nutrients in foods and stabilise pigment in wine during processing and storage (Dias et al. 2012; Du et al. 2012; Okilya et al. 2010). The difficulty in BITC degradation by both microwave and ultraviolet radiations increases with increasing concentration of BITC in

samples. BITC can react with 1, 2- benzenedithiol by a cyclocondensation reaction to form 1, 3- benzodithiole-2-thione (Zhang et al. 1992).

Use of biodegradation techniques for BITC degradation

Enterobacter cloacae strain P6A, which is highly pathogenic to human is the only organisms that had earlier been used to degrade benzyl isothiocyanate to benzylamine and hydrogen sulphide gas in the homogenates obtained from the papaya fruit and seed. *A. niger* is a commonly used organism in industrial fermentation for the production of various substances. It is a non-pathogenic filamentous fungus and is generally regarded as safe (GRAS) (Choe et al. 2012). *A. niger* has been reported to be involved in a number of biodegradation (Das

Fig. 5 The effect of benzyl isothiocyanate on glutathione concentration in *A. niger*

and Santra 2011). It is worthwhile to examine the potential role played by *A. niger* in the degradation of BITC, which is yet to be reported.

Organic isothiocyanates have been shown to react quantitatively with vicinal dithiols to form cyclic condensation products (Zhang et al. 1992). The cyclocondensation reaction of isothiocyanates gives a product, 1, 3-benzodithiole-2-thione. There was generally an increasing rate of transformation of BITC to 1, 3-benzodithiole-2-thione as its concentration increases (Fig. 3). As expected, all BITC concentration had no effect on day 0 because there was no growth of *A. niger* to metabolize it (Fig. 3). BITC compositions at lower concentrations of 0.136 and 0.408 nmol initially yielded higher growth rate of *A. niger* on day 1 compared with the compositions at higher BITC concentrations of 0.816, 1.36 and 2.72 nmol that showed no growth (Fig. 3). The reduced growth rate in the broth medium containing higher BITC concentrations may be attributed to the toxic effects of the high BITC. All the degradation compounds of glucosinolates, which are mainly thiocyanates, nitriles and isothiocyanates, exhibits toxic effects towards bacteria, fungi and insects (Wittstock et al. 2004). This toxicity level of isothiocyanates has a particular minimum inhibitory concentration. Interestingly, several pathogenic fungi have evolved the ability to detoxify chemical defense agents. For example, *Botrytis cinerea* is able to detoxify the tomato saponin (α -tomatine), which has been linked to the aggressiveness of this fungus (Quidde et al. 1998). Lower transformation of BITC to the product, 1, 3-benzodithiole-2-thione was observed on day 2 for all the BITC compositions. This is due to the reduction in the amount of BITC available within the broth medium as a result of increased microbial conjugation of the compound. Earlier studies on the biotransformation of naturally occurring isothiocyanates have shown that their metabolism takes place predominantly via the mercapturic acid pathway (Zhang 2004). A reduction in the amount of end product formed indicates that BITC was eliminated in the medium by *A. niger*. Isothiocyanate-GSH conjugates may have been formed, which are typically metabolized to yield cysteinylglycine-, cysteine- and *N*-acetylcysteine conjugates that are eliminated easily and concentrated in the urine. Research has shown that *N*-acetylcysteine conjugates and the intermediate metabolites of ITCs, which are collectively known as dithiocarbamates (DTCs) are unstable and easily dissociate to their parent ITCs (Conaway et al. 2001). This explains the sudden increase in the concentration of 1, 3-benzodithiole-2-thione formed on day 3 (Fig. 3).

The lower BITC compositions within 0.136 and 0.408 nmol increased GST activity on day 1 (Fig. 4). This may be due to the onset of *A. niger* growth seen in these compositions on day 1. However, it was the higher compositions of BITC that increased the activity of GST compared to the lower BITC compositions on day 3. BITC is an inducing agent of GST in living organisms. A positive correlation exists between

GST activity and the production of toxic compounds such as aflatoxin (Saxena et al. 1988). Thus, the increased GST activity can be attributed to high concentrations of toxic BITC. In addition, factors such as growth period and type of medium can also enhance GST activity (Saxena et al. 1988). Many animal studies have shown that phase II detoxification enzyme GST plays a key role in the elimination of isothiocyanate toxicity (Munday and Munday 2004). This is done by conjugating such compounds to GSH to excrete its derivatives from the body. GSTs are adopted for catalyzing the reaction of GSH with a variety of electrophiles (Munday and Munday 2004). The retention of GSH conjugate can also occur before compounds are delivered for excretion in bile. This can either function in the isolation of reactive conjugates or shift the equilibrium in favour of GSH conjugate. The latter has been observed in reactions involving organic ITCs. Expression of GST genes have been linked to the action of some fungal species such as *Issatchenkia orientalis*, *Phanerochaete chrysosporium*, *Yarrowia lipolytica*, *Mucor circinelloides*, *Schizosaccharomyces pombe*, *Aspergillus nidulans*, *Aspergillus parasiticus*, *Aspergillus flavus*, *Aspergillus fumigatus*, *Saccharomyces cerevisiae*, and *Cunninghamella elegans* (Krajewski et al. 2013).

All the BITC compositions did not affect GSH levels from day 0 – day 2 (Fig. 5). GST activity is often triggered in response to a stress that involves changes in GSH content, which is consumed in detoxification reactions protecting the cells (Dickinson and Forman 2002). All BITC compositions increased GSH levels just as *A. niger* increased in growth rate on day 3. This may be in response to the stress caused by increasing concentration of BITC. At higher concentrations, ITCs are recognized to cause oxidative stress (Zhang et al. 2005). A similar toxic compound, farnesol, depleted the concentration of intracellular GSH in *Candida albicans*, leading to the depletion of total GSH, oxidative stress and ultimately fungal death. It can also be deduced that the conjugation of BITC to GSH depleted the concentration of intracellular GSH of the *A. niger* administered with all the different BITC compositions on day 4 (Fig. 5). The antioxidant response elements (ARE) are stimulated by the conjugates, which in turn triggers the expression of a number of diverse detoxification and antioxidant-related genes (13). The transport of conjugates from the cell causes a swift and significant depletion of GSH. The induction of the genes ultimately leads to an increase in intracellular GSH beyond the average level in order to replete GSH levels (Zhang et al. 2005). The induction of genes that activates ARE is also to enable the uptake and metabolism of more ITCs. ITC uptake is positively associated with its concentration and the period of its availability. ITC uptake is also boosted in the presence of GSTs, which supports GSTs and isothiocyanate conjugation. In mammals, isothiocyanates are known to be metabolized via the mercapturic acid pathway (Shapiro et al. 2001). In the first step, they are

conjugated with glutathione GSH, a reaction that can be catalyzed by mammalian GSTs in vitro. Glutathione (L- - glutamyl-L-cysteinylglycine) is a non-protein thiol that is in abundance in mammalian cells. As a strong nucleophile, GSH can react naturally with many electrophiles even without the catalysis of GSTs (Traka and Mithen 2009). Thus, high intracellular levels of GSH in mammalian cells may exclusively constitute a vital line of defense against reactive electrophiles, such as isothiocyanates, and oxidative stress (Dickinson and Forman 2002). Glutathione acts as a transporter of isothiocyanates since the conjugation is reversible, and isothiocyanates can be transferred from glutathione to another endogenous nucleophile (Temmink et al. 1986). Isothiocyanates and GSH without any enzymatic support react quickly with each other. They are quickly accumulated in the cell, generally in the form of GSH conjugates. The GSH level of *A. niger* increases with increasing levels of BITC, in a manner similar to the increased in GST activity in response to increasing BITC concentration reported in this work. This GSH level was depleted with prolong exposure time of *A. niger* to BITC. Bio-transformation of BITC to a more soluble and excretable product by *A. niger* can be attributed to the GSH depletion.

Conclusions

The use of both microwave and ultraviolet radiations, and the involvement of *A. niger* in the bio-degradation of BITC has been reported for the first time in this work. Microwave radiation was most effective since it facilitates rapid BITC degradation, but ultraviolet radiation may provide the most economical benefits for reducing the BITC in papaya oil. Thus, the dietary consumption level of papaya seed oil can be improved with the application of both microwave and ultraviolet radiations, and by taking advantage of the effectiveness of *A. niger* in degradation of BITC.

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Competing interests Authors have declared that no competing interests exist.

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